A NEW MICROASSAY FOR CHLORITE

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In the last two years there has been a significant increase in the amount of interest being focussed on chlorine oxyanion contamination, especially perchlorate. Due to the unique chemical stability and high solubility of perchlorate, microbial reduction has been identified as the most practical means of remediating this contaminant, however, until recently very little was known about microorganisms involved in microbial (per)chlorate reduction. Recent studies in our lab have demonstrated that the ubiquity and diversity of (per)chlorate-reducing bacteria (ClRB) is far greater than was previously assumed. As part of these studies we demonstrated that chlorite dismutase is a central enzyme in the reductive pathway of perchlorate that is common to all ClRB which dismutates chlorite into chloride and molecular oxygen. As a continuation of these studies we have developed a microassay for the detection of chlorite. The assay is based upon the enzymatic reaction between chlorite and horse radish peroxidase (HRP). The dye, o-dianisidine acts as an electron donor in reaction with the end product of the HRP. Preliminary data suggests that the chlorite is converted to chlorine dioxide and chloride which is comparable to the results obtained in previous studies. The assay was conducted by incubating 10µL of a solution containing chlorite (typically 5mM) with 2mL of a solution containing horse radish peroxidase (1U/mL) and the dye o-dianisidine for a specific time at a specific temperature. The o-dianisidine was oxidized by the ensuing reaction, forming a yellow product the absorbance of which was read at 450nm, using a Spectronic Genesys 8 spectrophotometer (Spectronic Instruments, Inc., Rochester, NY).

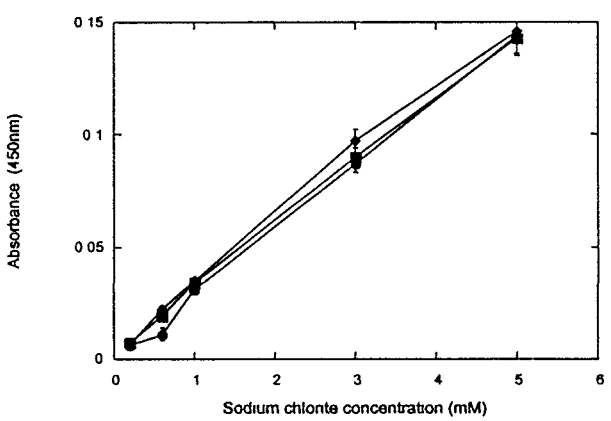

Figure 1. Linearity and reproducibility of chlorite microassay. Results depicted are of triplicate determinations of three sets of separate standards.

The assay was optimized for time, temperature and pH. It performed maximally at room temperature (22°C) with an incubation time of 20 minutes in 100mM phosphate buffer at pH 7.3. Under these conditions the assay was found to be linear over the concentration range of 0.02mM – 6.0mM chlorite, and to be highly reproducible as depicted in Fig. 1.

Potential interference from common environmental ions, such as nitrate (NO_3^-), nitrite (NO_2^-), sulfate (SO_4^{2-}), sulfide (S^{2-}), thiosulfate ($S_2O_3^{2-}$), dithionite ($S_2O_4^{2-}$), and ferric iron (Fe^{3+}) was determined. In addition, interference from other ions of chlorine such as perchlorate (ClO_4^-), chlorate (ClO_3^-), hypochlorite (ClO^-), or chloride (Cl^-) was also investigated. The results of these experiments indicated that the assay was largely unaffected by (per)chlorate, chlorate, chloride, nitrate, nitrite, sulfate, and ferric iron, but was inhibited by strong reductants such as sulfide, thiosulfate, and dithionite to varying degrees (Fig. 2). The strong oxidant, hypochlorite, interfered competitvely with the chlorite for the HRP (data not shown).

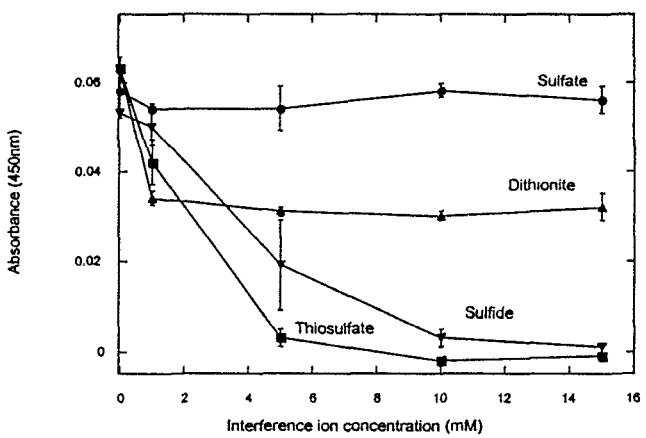

Figure 2. The effect of the presence of sulfur ions on the microassay for chlorite. Results depicted are triplicate determinations at each interfering ion concentration.

This study has resulted in the development of a versatile, quick, reliable, and cheap microassay for chlorite. This assay has been effectively used by our group during the purification of a chlorite dismutase enzyme (CD) from the dissimilatory (per)chlorate-reducer, *Dechlorimonas agitatus* strain CKB. Previous similar studies relied on the measurement of oxygen production resulting from chlorite dismutation to identify CD active fractions during the purification protocol. These techniques were cumbersome and unreliable, requiring large volumes of the active fractions. The advantage of the application of the new microassay technique is speed, reproducibility, and the low volume (20 μl) of active fraction required.

The current method available for the analytical measurement of chlorite is high pressure liquid ion chromatography, coupled to a conductivity detector which has the advantage of small sample size for analysis (10-25 μL), but it is expensive and cumbersome requiring suppressed conductivity for sensitive measurements (4nmol). The microassay described here has many advantages over ion chromatography including

- No specialist equipment is required, except a spectrophotometer which is a common piece of equipment in most laboratories.
- Sample size is similar to that required for HPLC-IC
- The limit of detection is approximately 0.2nmol with an upper limit of 50nmol, which is on the same order of magnitude as the HPLC-IC method.
- It is quick and reliable requiring a total analysis time of 30 minutes.

Future work on this project includes developing a field assay for the "on the spot" analysis of chlorite contaminated samples.